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SPECIFICATION

TITLE OF INVENTION

(This application claims priority under **35 USC 119(e)**. This application is a continuation of and claims benefit of U.S. PTO Ser. No. **60/263,273**.)

[0001] Automated method, automated process control methodology, process control interface, and automated apparatus for pancreatic islet isolation (separation) and processing utilizing microprocessor controllers and computer control of the process variables and automated apparatus including real-time process data acquisition.

CROSS-REFERENCE TO RELATED APPLICATIONS "Not Applicable"

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT "Not Applicable"

REFERENCE TO A MICROFICHE APPENDIX "Not Applicable"

FIELD OF THE INVENTION

[0002] The present invention relates to the isolation of pancreatic islets for transplantation into diabetic patients. The invention presents Advanced Islet Separation Technology, an automated method of islet isolation (separation), automated process control methodology, process control interface, and automated apparatus to separate and process pancreatic islets in physiologic process solution utilizing microprocessor control and computer control of the process variables and automated apparatus. The invention may be uniformly applied to islets harvested from animals and mammals either transgenic or non-transgenic.

BACKGROUND OF THE INVENTION

[0003] The islets of Langerhans, endocrine tissue containing insulin producing beta cells, represent about one to two percent of the total mass of the human pancreas. Separation or isolation of the islets from the connective matrix and remaining exocrine tissue is advantageous and beneficial for laboratory experimentation and transplantation purposes. Islet transplantation is a most promising and minimally physiologically invasive procedure for treatment of type I diabetes mellitus. Transplanting islets rather than complete pancreatic tissue has the distinct advantages of ease of transplantation, and the elimination of the pancreatic exocrine function of the donor tissue involving secretion of digestive enzymes. Liberating islets from pancreatic exocrine tissue is the initial and crucial step that influences islet transplantations. The important objective in islet isolations is to provide sufficient numbers of viable functional and potent islets for transplantation.

[0004] Early methods of islet separation involved chopped pancreatic fragments mixed with collagenase and incubated around 37 degrees C, D. Scharp, World Journal of Surgery **8**:143-151, 1984, incorporated herein by reference. Collagenase breaks down and digests pancreatic tissue, freeing islets, however, prolonged exposure to proteolytic enzymatic digestion destroys the initially separated islets. If the separation is stopped to protect the early released islets, too few islets are freed from the surrounding exocrine tissue. Islet isolation in small mammals was improved utilizing mechanical distension of the pancreas, increasing islet yield through mechanical separation of pancreatic tissue. Yet, with larger mammals this technique did not allow sufficient numbers of islets to be separated from a single donor for transplantation. Further improvements in islet separation included perfusion of the pancreas with collegenase via ductal distention causing mechanical disruption of exocrine tissue, U. S. Patent 5,322,790, D. Scharp, June 21,1994, incorporated herein by reference. In addition, tissue disintegration resulting in islet separation by mechanical disruption of the pancreas has been effected by perfusion of the pancreas with an enzyme containing solution while in a chamber with solid spheres and contacting (beating) the pancreas with the spheres, either by hand or by motorized repetitive motion. This is mentioned in U. S. Patent 5,853,976, Hesse, et al., December 29, 1998, incorporated herein by reference, and is a component of the standard isolation technique currently practiced. Recently, islets have been isolated using enzymatic digestion and sound waves to rupture pancreatic tissue, U. S. Patent 5,879,939, Gray et al., March 9, 1999, incorporated herein by reference. Islets have also been separated using both a warm and cold

digestion stage, in which physiologic solutions other than Hanks Balanced Salt Solution (HBSS) have been utilized for the second cold digestion stage, with increased islet yield and functionality when compared to the islets separated utilizing HBSS for the second cold separation, U. S. Patent 5,919,703, Mullen, et al., July 6, 1999, incorporated herein by reference.

[0005] While larger scale islet separation from human pancreases has become possible with advances in technology, the previously cited techniques fall short in terms of efficiency, and are inadequate for scale up or mass production in which many donor pancreases are processed at different research and transplantation centers, medical facilities, or commercial locations. In consideration of the lack of donor pancreases, current islet isolation techniques are also inadequate to continuously and repetitively batch process porcine pancreases, or pancreases from animals or mammals, transgenic or non-transgenic, to produce islets for xenotransplantation. In the previously referenced techniques and patents there exist limitations in the methodologies that may significantly affect the outcome of the islet separation process.

[0006] Collegenases, metalloendoproteinases that cleave collagen into smaller peptide fragments, are zinc-containing enzymes that require divalent calcium as a cofactor for stabilization and optimal activity. Using traditional collegenases detrimentally affects pancreatic digestion due to the impurities present in the collegenase solutions. Traditional collagenase preparations are concentrated from bacterial (Clostridium histolyticum) culture supernatants. Roche, a manufacturer of molecular biochemicals, states that such collegenase preparations are heterogeneous, containing as many as 30 different enzymes, pigments, cellular debris, and endotoxins. The most significant liabilities of traditional collegenase are variability and endotoxin levels. In traditional collagenase, the primary enzymatic constituent is collegenase, classes I and II, described by Bond and Van Wart, Biochemistry, **23**:3077, 1984, and Biochemistry, **23**:3085-3091, 1984, incorporated herein by reference. Other proteases found include neutral protease, clostripain, elastase, trypsin, and aminopeptidase. Non-proteolytic enzymes isolated from collagenase preparations include hyaluronidase, galactosidase, acetyl glucosaminidase, phospholipase, fucosidase, and neuraminidase.

[0007] Endotoxin is associated with a number of cellular events including cell activation with subsequent cytokine secretion, and programmed cell death (apoptosis). Endotoxin exposure is postulated to cause a loss of transplanted pancreatic islets, Vargas et al., Transplantation, **65(5)**: 722-727, March 15, 1998, incorporated herein by reference. They have demonstrated that supernatants generated during islet separation were able to induce certain inflammatory

cytokines in the islets during the separation process. They postulate that endotoxins and locally induced cytokines accompanying the transplanted islets activate the endothelium and promote lymphomonocytic infiltration of the transplanted islets and surrounding liver tissue. Jahr et al., J. Mol. Medicine (Berlin), **77(1)**:118-120, January 1999, incorporated herein by reference, suggest that endotoxin-induced early inflammatory reactions may inhibit the function and survival of isolated cells or cell aggregates after transplantation. Eckhardt et al., J. Mol. Medicine (Berlin), **77(1)**:123-125, January 1999, incorporated herein by reference, have determined that islet xenograft survival increased in endotoxin free conditions. Clearly, endotoxin and accompanying cellular reactions may cause non-function and rejection of transplanted islets.

[0008] Alternatively, Roche manufactures non-traditional purified collegenase blends of collegenase I and II (including either neutral protease dispase or neutral protease thermolysin) for pancreatic tissue dissociation, under the product name of Liberase®. Liberase prepared for human islet separation incorporates thermolysin. Neutral protease acts synergistically with collegenase during intercellular matrix digestion. During Liberase enzyme production, collagenase isoenzymes are purified from raw collagenase by a process that removes greater than 99% of the endotoxin present in the collagenase raw material. Enzyme purification also removes non-enzymatic components and bacterial cellular debris that may be toxic to islets. Collagenases I and II are purified to greater than 95% homogeneity. Roche proof data shows that Liberase contains on average less than 10 Endotoxin Units (EU) per milligram (mg), while traditional collegenase preparations contain far greater than 1000 EU/mg and may have as much as 13000 EU/mg. Liberase represents a defined product for tissue dissociation, U.S Patent 5,952,215, Dwulet, et al., September 14, 1999, incorporated herein by reference. Linetsky et al., Transplant Proc., **30(2)**:345-346, March 1998, incorporated herein by reference, demonstrated that the use of Liberase enzyme improved human islet yield, compared with traditional collagenase. Examination of absolute islet number, islet number per gram of pancreas, islet equivalent number, and islet equivalent number per gram of pancreas indicated that Liberase enzyme improved islet yield. Liberase gives maximal tissue dissociation performance with minimal endotoxin complicity when compared with traditional collegenases.

[0009] Apoptosis can also be initiated by biochemical factors other than endotoxin. Nitric oxide and its metabolites are known to cause cellular death from nuclear damage (apoptosis), U. S. Patent 5,834,005, A. Usula, November 10, 1998, incorporated herein by reference. Nitric oxide is a recognized multifunctional mediator that is produced by and acts on various cells, and

participates in inflammatory and autoimmune-mediated tissue destruction, U. S. Patent 5,919,775, Amin et al., July 6, 1999, incorporated herein by reference. The group of enzymes known as nitric oxide synthases catalyzes nitric oxide production. Nitric oxide synthase (NOS) is expressed in mammalian cells. Utilizing cofactors in the presence of oxygen, it catalyzes the mixed functional oxidation of L-arginine to L-citrulline and nitric oxide, by removing a guanidino nitrogen from L-arginine to form nitric oxide. Interleukin-1 (IL-1) has been shown to induce the expression of the cytokine inducible isoform of nitric oxide synthase in pancreatic islets. The production of nitric oxide has been proposed to be the effector molecule that mediates IL-1's inhibitory effects on islet function, U. S. Patent 5,837,738, Williamson et al, November 17, 1998, herein incorporated by reference.

[0010] Yet, the deleterious effects of nitric oxide on islet cells can be alleviated by a variety of means. Inhibitors of nitric oxide synthase have been identified. Nitric oxide synthase (NOS), and subsequently nitric oxide, can be inhibited by derivatives of L-arginine, the natural substrate of nitric oxide synthase. These include methyl-, dimethyl-, or amino-substituted guanidines. These inhibitory compounds are also chemically known as aminoguanidinie, N, N'-diaminoguanidine, methylguanidine and 1, 1-dimethylguanidine (U. S. Patent 5,837,738 and U. S. Patent 5,919,775, both previously incorporated herein by reference). Nitric oxide production can also be inhibited by 2,4-diamino-6-hydroxy-pyrimidine, a compound that interferes with the activity of a cofactor of inducible nitric oxide synthase. Antibiotic tetracycline also inhibits nitric oxide synthase, thus preventing the formation of nitric oxide, as do doxycycline, and minocycline, a semisynthetic tetracycline (U. S. Patent 5,919,775, previously incorporated herein by reference). Nitric oxide can also be inhibited by nitric oxide scavengers such as cysteine, and other sulfated compounds such as dextran, heparin, and cystine, U. S. Patent 5,834,005 (previously incorporated herein by reference). Alternatively, sparging with an inert gas such as helium can effectively control and eliminate the dissolved oxygen concentration in the islet containing physiologic process solution, thereby hindering the production of nitric oxide via reduction and catalytic oxidation of L-arginine by NOS and cofactors. In combination with oxygen removal from the process solution, cysteine, dextran, heparin, and cystine also inhibit nitric oxide formation that results from relative states of islet hypoxia. Nitric oxide inhibition and scavenging improves islet survival and secretory function. It is certainly beneficial to control or inhibit the formation of nitric oxide in islets and the islet containing physiologic process solution.

[0011] The method of mechanical tissue dissociation with glass marbles, steel balls or other sufficiently dense and solid objects, either by hand or with mechanical shaking (Ricordi shaker), may cause tissue damage and trauma to islets resulting from excessive shear stress during the separation process. While repetitive mechanical agitation and contacting the pancreas with solid objects effects tissue disruption aiding enzymatic digestion, such current practices in standard isolation techniques are subjective, and vary between research facilities and transplantation centers.

[0012] Although sonication has been employed to aid pancreatic tissue digestion, one certain limitation in this technique is the 'static' water bath that the 'bagged' pancreas is placed in. Interestingly, this technique is continued until the pancreas appears 'cracked', yet, no mention of the internal temperature of the pancreas is noted. Static digestion by any method offers no means of forced-convective heat transfer to maintain a constant processing temperature (cooling during sonication) of the digesting pancreas or the resulting tissue suspension, by the process solution. It is possible that the internal temperature of a bagged pancreas in such a static system exceeds 37 to 40 degrees C, a temperature considered optimal for functional enzymatic digestion, yet, minimal in thermal shock and deactivation of islets due to elevated temperature. A statically digested pancreas in a bag offers no opportunity to maintain a controlled internal pancreatic temperature. This method presents no opportunity to dilute the tissue suspension, which precludes a real-time method to control, deactivate, or inhibit the digestive enzymes in the processing solution, during islet separation and processing, in the dilution and collection phase.

[0013] At the XVIII International Congress of the Transplantation Society, August 27 - September 1, 2000 in Rome, Italy, advances in pancreatic islet cell transplantation procedures were reviewed and discussed. Existing limits of transplantation and novel approaches to achieving tolerance were evaluated. It was noted that success of recent transplantations (Edmonton Protocol) might certainly be due to the use of immunosuppression that was not toxic to beta cells. Avoiding the use of corticosteroids, induction therapy with anti-IL-2 antibody, and low-dose tacrolimus and sirolimus maintenance were undoubtedly key factors in non-rejection and continued islet tolerance. The quality of the purified islets also contributed to the success of the transplantations, yet, acquired by tedious and laborious manual methods lacking in process control methodology and neglecting important process variables. Current challenges were also assessed, specifically, the standardization of islet separation technology, and the need to

development a standardized, reproducible, and automated method to separate and produce high-quality islet cells.

[0014] Presently there exists no process control methodology of the islet separation process that takes into account crucial process variables that may be controlled to optimize islet isolation while standardizing and automating the islet separation process. Importantly, there are separation and processing variables that have been neglected and omitted which compromise the reproducibility and repeatability of the islet separation process from location to location. Objectively applying advanced process control methodology and automating the islet isolation process with process control technology can optimize the islet separation process.

BRIEF SUMMARY OF THE INVENTION

[0015] The present invention relates to the application of advanced control methodology and process control technology, incorporating an automated method of islet separation (isolation) and processing utilizing microprocessor and computer control and software programming, to control the process variables and interface the process control methodology to the automated islet separation apparatus, via an electrical (electronic) interface and a graphic user interface, and includes real-time data acquisition (DAQ) and recording of the process variables. Process variables that influence the islet separation process have been identified and incorporated into the automated method of islet separation by integrating automated control of the process variables through the process control interface to the islet separation apparatus, utilizing an analog and digital (A/D) electrical (electronic) interface with feedback from and control of the islet separation apparatus. The identified process variables that influence the islet separation process are the process solution, the process temperature (T), the process flowrate (F), the percent hydrogen (pH), the dissolved oxygen (DO) concentration, the dissolved nitric oxide (NO) concentration, the dissolved carbon dioxide (CO2) concentration, the digestive enzyme (PE) activity, the endotoxin (E) concentration, the antibiotic (A) concentration, and the pressure (P) of the islet containing physiologic process solution. By controlling the variables that influence the reproducibility and repeatability of the islet separation process, the present invention is superior to the currently employed and subjectively standard islet isolation techniques, which neglect process control of the important and essential process variables.

[0016] In the preferred application of the automated method of islet separation utilizing the automated islet separation apparatus, a single researcher performs the islet isolations. Once a

pancreas is suitable prepared and made ready for islet separation processing (U. S. Patent 5,322,790, U. S. Patent 5,853,976, both previously incorporated herein by reference), the researcher uses the automated islet separation method to start the automated islet separation apparatus with a single command via the electrical (electronic) and graphic user interface, initiating the fill, circulate and rinse cycle. After the initial rinse cycle and the rinse solution is discarded, the islet separation apparatus again fills with physiologic solution and the method and apparatus will then pause in operation and wait for the pancreas to be loaded in the dynamic flow digestion chamber.

[0017] After pancreas addition, once the dynamic flow digestion chamber is sealed, the researcher restarts the automated method of islet separation and the automated islet separation apparatus with a single command via the graphic user interface, and monitors the process via the electrical (electronic) and graphic user interface (computer display). Once the automated islet separation method and the islet separation apparatus are restarted, the islet separation apparatus will automatically adjust, attain, maintain, regulate, and control the desired, preset, and programmed values of the process variables during the digestion, separation, and circulation phase. The process variables are the programmed (setpoint) process temperature (T), the programmed (setpoint) process flowrate (F), the programmed (setpoint) percent hydrogen (pH), the programmed (setpoint) dissolved oxygen (DO) concentration, the dissolved nitric oxide (NO) concentration, the dissolved carbon dioxide (CO2) concentration, the programmed (setpoint) digestive enzyme (PE) activity, the endotoxin (E) concentration, the antibiotic (A) concentration, and the pressure (P) of the islet containing physiologic process solution.

[0018] At the end of the digestion, separation, and circulation phase of islet processing, when the desired extent of islet separation is achieved, a single command from the researcher cycles the automated islet separation apparatus into the dilution and collection phase. During the dilution and collection phase, methodology is employed to control, deactivate, and inhibit the digestive effects of proteolytic enzymes and tissue dissociating agent collegenase (Liberase), protecting the islets from unnecessary degradation during dilution and collection. Quite simply, during dilution and collection, collegenase can be inhibited by the addition of chelators of divalent cations such as citrate, EDTA, or EGTA. Metalloendoproteinase activity can also be inhibited by cysteine, tetracycline, and doxycycline, Smith Jr. et al, Annals NY Acad Sci **732**:436-438, 1994, incorporated herein by reference. Throughout the dilution and collection phase, the fundamental process variables are still controlled at the programmed (setpoint) values.

During this phase of islet processing, the islet separation apparatus will dilute and autocollect the islet containing physiologic process solution via the automated islet autocollector.

[0019] The researcher may at any time alter, control, stop, and restart the process, when necessary, due to unforeseen disturbances. While the islet separation process is fully automated, it is still an automated batch process fully under the researcher's control via the electrical (electronic) and graphic user interface. Ultimately, the process will need no manual intervention and the entire separation process, after the loading of the pancreas into the dynamic flow digestion chamber, will proceed by the automated method. The automated method of islet separation, the automated islet separation apparatus, and the process variables are microprocessor and computer controlled, and the entire islet separation process starting with the fill, circulate and rinse cycle, the pancreas addition proceeding through digestion, separation and circulation, and ending with the dilution and collection phase may be accomplished with either start stop commands to process controllers and pumps and or three single keystrokes or mouse clicks, or touch screen commands.

[0020] During automated islet separation processing, specifically digestion, separation and circulation, the islet containing physiologic process solution is monitored via autosampled aliquots of the process solution. Depending on the level of sophistication required by the individual researcher, research facility, or transplantation center, the extent of pancreatic digestion and islet separation monitored in real-time may be accomplished either manually via autosampled aliquots, staining, and image analysis software, or automated on-line via integration of a flow through sample cell, autosampling, staining, and image analysis software. Importantly, during the islet separation, the endotoxin concentration of the islet containing physiologic process solution is monitored, recorded and controlled in real-time via the same autosampled aliquot.

[0021] Process control and data acquisition of the automated method of islet separation and the automated islet separation apparatus is accomplished with microprocessor controllers and a windows based (Win98) microprocessor computer (PC) operating in the LabView graphical software-programming environment (G), for example. The microprocessor computer (PC) is equipped with analog and digital solid-state electrical (electronic) input output control boards that are electrically (electronically) interfaced to various analog and digital input output control hardware via sensors and electrical (electronic) connections. Islet separation process data are collected in real-time and the researcher is presented with an electrical (electronic) and graphical

interface that contains an interactive process flowsheet displaying system status and processing data, which accepts user input and feedback to the process. Pumps, valves, thermocouples, sensors, probes, and transducers may be located on a graphical process flowsheet (computer display) and correspond to their actual physical location on the automated islet separation apparatus. The software may be programmed to automatically control the automated islet separation apparatus and the process variables at the programmed set points; yet, the researcher may at any time manually override the process. Islet separation process data are acquired during islet isolation from the process sensors and process controllers and microprocessor computer (PC) and logged in a data file for post-processing analysis, quality assurance, validation, and regulatory purposes.

[0022] Analog output process sensors including thermocouples, dissolved oxygen sensor, percent hydrogen probe (sensor), nitric oxide sensor, carbon dioxide sensor, endotoxin sensor, process pump tachometer (flowrate), and pressure (transducer) sensor are interfaced to signal conditioning input modules which input process data through the electrical (electronic) interface to the microprocessor controllers and the microprocessor computer (PC). The process pump speed (flowrate) is controlled by analog output from the microprocessor computer (PC) through a signal conditioning output module and the electrical (electronic) interface. The process temperature (T), the process flowrate (F), the percent hydrogen (pH) concentration, the dissolved oxygen (DO) concentration, the dissolved nitric oxide (NO) concentration, the endotoxin (E) concentration, the proteolytic enzyme (PE) activity, the antibiotic (A) concentration, and the pressure (P) are controlled by feedback from process sensors and analog and digital output from the microprocessor controllers and the microprocessor computer (PC) via analog and digital and solid state relays (SSR) through the electrical (electronic) interface. Analog and digital output from the microprocessor computer (PC) via the analog-digital (A/D) interface through the electrical (electronic) interface is used to activate electric solenoid valves controlling the process solution flow and flow direction. The process solution flow and direction of fluid flow of the automated islet isolation apparatus may be operated under manual and or electrical control via the electrical (electronic) and graphical interface.

[0023] Other operating systems such as WinXP, Win2000, WinNT, Win95, Win3.1, Linux, Warp, OS-2, or Mac OS for example, and other software-programming environments, Fortran, Pascal, C, C+, C++, Basic, Visual Basic, Java, HTML, or VRML for example, as well as programmable logic controllers (PLC) and or programmable microprocessors (non PC) may be

utilized to interface the automated control methodology and automated islet separation apparatus. Microprocessor controllers and or microprocessor computer (PC) and or programmable logic controller (PLC) may also be employed for automating the motion control of the autosampler, autocollector, and dynamic flow digestion chamber. General-purpose input output boards (GPIB) or RS-232 (RS 485) inputs and outputs may also be employed to control the operation of the automated islet separation apparatus including data acquisition.

[0024] It is the object of this invention to present Advanced Islet Separation Technology and apply the technology to pancreatic islet separation (isolation) and processing. Advanced Islet Separation Technology incorporates advanced control methodologies to control and monitor the critical process variables that affect the reproducibility and repeatability of islet separation processes from location to location.

[0025] Specifically, the present invention utilizes process control methodology and applies it to pancreatic islet isolation, uniquely incorporating an automated method, automated control methodology, process control interface, and an automated apparatus to separate and process pancreatic islets in physiologic process solution.

[0026] More particularly, this invention utilizes process variable setpoints employing microprocessor controllers, and microprocessor computer control and software programming (code), to automate and interface the process control methodology, on-line in real-time, to control the process temperature (T), the process flowrate (F), the percent hydrogen (pH) concentration, the dissolved oxygen (DO) concentration, the dissolved nitric oxide (NO) concentration, the dissolved carbon dioxide (CO2) concentration, the digestive (proteolytic) enzyme (PE) activity, the endotoxin (E) concentration, the antibiotic (A) concentration, and the pressure (P) of the islet containing physiologic process solution during islet separation and processing, via an analog and digital electrical (electronic) interface, with feedback from the process sensors and control of the automated islet separation apparatus by the microprocessor controllers and computer and software code.

[0027] This invention provides on-line in real-time, process data acquisition and recording of the process variables during islet separation and processing, via an analog and digital electrical (electronic) interface and input from the process sensors in the islet separation apparatus.

[0028] This invention incorporates real-time image analysis to determine the extent of pancreatic digestion and islet separation to control the digestion time, utilizing image analysis software. Real-time image analysis may be accomplished either by discrete autosampling and

manually staining utilizing microscopy and image analysis, or automated via a flow through sample cell, and staining utilizing discrete on-line image analysis, via an analog and digital electrical (electronic) interface and input to the islet separation apparatus.

[0029] This invention also provides a modified and improved temperature controlled, dynamic flow digestion chamber, incorporating forward fluid flow and reversing fluid flow, and rotary motion (or linear motion, or eccentric motion) with self-contained sonic transducers with sonication applied to the dynamically flowing islet containing processing fluid, to aid tissue digestion via an analog and digital electrical (electronic) interface and input to the islet separation apparatus (dynamic flow digestion chamber).

[0030] Provided by this invention are methods to control, deactivate, and inhibit the digestive effects of the proteolytic enzymes utilized to digest pancreatic connective tissue (collagen) at the end of the digestion, separation and circulation phase, during the dilution and collection phase.

[0031] This invention further provides a method to control and monitor in real-time, the nitric oxide concentration present in the islet containing physiologic process solution during islet separation and processing.

[0032] This invention further provides a method to monitor and control in real-time via discrete analysis, the endotoxin concentration present in the islet containing physiologic process solution during islet separation and processing.

[0033] This invention further provides a method to monitor and control in real-time, the carbon dioxide concentration present in the islet containing physiologic process solution during islet separation and processing.

[0034] The above is a brief description of the advantages of the present invention. The features, embodiments, and advantages of the invention will be apparent to those skilled in the science from the accompanying drawings, following description, and appended claims.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING

[0035] FIG. **1** is a process flowsheet of the automated islet separation apparatus showing the various components of the automated islet separation apparatus and the microprocessor controllers and microprocessor computer interfaced to the automated apparatus according to one preferred embodiment of the invention.

[0036] FIG. **2** is a schematic and block diagram of the automated islet separation apparatus showing the interaction of the components of the automated apparatus including the analog and

digital electrical (electronic) interface and the microprocessor controllers and the microprocessor computer according to one preferred embodiment of the invention.

[0037] FIG. **3** is a flowchart showing the sequence of operations of the automated islet separation method in the order performed by the automated islet separation apparatus according to one preferred embodiment of the invention.

[0038] FIG. **4** is a process control valve logic diagram of the automated islet separation apparatus showing the valve logic of the automated method according to one preferred embodiment of the invention.

[0039] FIG. **5** is a separate view of the dynamic flow digestion chamber showing the direction of forward fluid flow through the digestion chamber according to one preferred embodiment of the invention.

[0040] FIG. **6** is a separate view of the dynamic flow digestion chamber showing the direction of reverse fluid flow through the digestion chamber according to one preferred embodiment of the invention.

DETAILED DESCRIPTION OF THE INVENTION

[0041] The invention is directed at an improved and automated method of islet isolation (separation), employing automated process control methodology, a process control interface, and automated apparatus to separate and process pancreatic islets in physiologic process solution utilizing microprocessor controllers and microprocessor computer control (PC) of the process variable setpoints and automated apparatus, including real-time process data acquisition. The invention is also directed at a dynamic flow digestion chamber incorporating forward fluid flow and reversing fluid flow, rotary motion (or linear motion, or eccentric motion) with self-contained sonic transducers incorporated in the dynamic flow digestion chamber with sonication applied to the dynamically flowing islet containing processing solution.

[0042] Figure **1** illustrates a flowchart of the interaction of the various components of the automated islet isolation apparatus according to one preferred embodiment of the invention. As shown in Figure **1**, the physiologic process solution flows from feed tank **101** incorporating heater **102** through process tube **103** through process valve **104** through process tube **105** through process pump **106** through process tube **107** through process heat exchanger **108** through sensor block **114** that contains process sensors **109** dissolved oxygen sensor, **110** nitric oxide sensor, **111** pH sensor, **112** temperature (thermocouple) sensor, **113** pressure (transducer) sensor, **160**

endotoxin sensor, **161** carbon dioxide sensor, and in the forward flow direction through process valve **116** through rotary coupling **118** through the dynamic flow digestion chamber **120** incorporating temperature (thermocouple) sensor **121** and process heater **123** through rotary coupling **124** through process valve **180** through process valve **126** through process tube **128** through process valve **132** through process heat exchanger **133** through temperature (thermocouple) sensor **134** through process tube **138** through process valve **139** into collection vessel **151** on stirring plate **150** through process tube **152** through process valve **154** through process tube **155** returning to the feed tank **101** during the circulation and rinse phase of islet processing. After pancreas addition to the dynamic flow digestion chamber, during the digestion, separation, and circulation phase of islet processing the physiologic process solution flows from process tube **152** through process valve **153** again through process tube **105** forming a process loop.

[0043] Figure **1** also illustrates according to one preferred embodiment of the invention the process sensors (previously described) and the microprocessor controllers (setpoint enabled), **200** temperature controller, **201** pH controller, **202** dissolved oxygen controller, **203** endotoxin neutralizing protein (ENP) controller, **204** proteolytic enzyme (collegenase) neutralization (PEN) controller, **205** nitric oxide controller, and **206** carbon dioxide meter, interconnected by electrical (electronic) wiring **156**, the analog and digital and connector block interface **157**, and microprocessor computer **159** utilizing **157** interconnected by electrical (electronic) wiring **158** , the oxygen control valve **146**, the oxygen tank **147**, the helium tank **148**, the helium control valve **149**, the acid reservoir **140**, the acid pump **141**, the acid addition tube **145**, the base pump **142**, the base reservoir **143**, the base addition tube **144,** the endotoxin neutralizing protein (ENP) pump **210**, the ENP solution reservoir **211**, the ENP addition tube **214**, the (digestive) proteolytic enzyme neutralization (PEN) pump **212**, and the PEN solution reservoir **213**, the PEN addition tube **215**, which are employed to control the chemical character of the process solution at the setpoints during islet isolation and separation processing. Periodic sampling of the process solution is accomplished via sampling process valve **135** and the auto-sampler **136**. When the pancreas has been sufficiently digested, auto-collection of the islets and the process solution is accomplished when process solution flows through process valve **131** through process tube **130** and into the auto-collector **129**.

[0044] Figure 2 illustrates a schematic and block diagram of the interaction of the various components of the automated islet isolation apparatus, electrical connections and wiring according to one preferred embodiment of the invention. Electrical process connections **156** interface the process sensors **109, 110, 111, 112, 113, 121, 134, 160, 161** (previously described in Fig. 1), the microprocessor process controllers **200, 201, 202, 203, 204, 205** (previously described in Fig. 1), and the microprocessor process meter **206**, (previously described in Fig. 1) through the analog and digital and connector block interface **157** to the automated islet separation apparatus. A microprocessor computer (PC) **159** is employed to control the electric (solenoid) process valves **104, 115, 116, 117, 125, 126, 127, 131, 132, 135, 137, 139, 146, 149, 153, 154, 180** (previously described in Fig. 1) and to record the processing data via real-time process data acquisition (DAQ - sensor output from the sensors and microprocessor process controllers and microprocessor process meters) through electrical (electronic) process connections **158** and the analog and digital and connector block interface **157** incorporating input modules. The microprocessor computer **159** consists of the program memory **159(A)** random access memory (RAM) and read only memory (ROM), stored by a hard-drive (HD) and or erasable programmable read only memory (EPROM), software code **159(B)** stored by either RAM, ROM, EPROM, or HD, and user interface **159(C)** incorporating keyboard, mouse, interconnection cables and a numerical (electrical [electronic]) and graphical display (computer monitor) **159(D)**.

[0045] Figure **3** illustrates a flowchart illustrating the automated method of islet isolation according to one preferred embodiment of the invention. Initially the automated islet isolation process is started **301**, the automated islet separation apparatus fills with physiologic process solution **302**, the process solution is circulated and the interior of the islet separation apparatus is rinsed **303**, the process solution is drained **304**, and the apparatus is refilled **305** with fresh physiologic process solution after which the automated method and automated apparatus pause in operation (flow) **306** while pancreas addition **307** to the digestion chamber takes place. The method and apparatus are restarted **310**, initiating pancreatic digestion, separation and circulation **309** of islets and processing fluid, while real-tine process data acquisition (DAQ) **308** is executed. During **309**, autosampling **312** is executed and the method and apparatus may be paused **311** at any time. When sufficient numbers of islets have been liberated and separated from the pancreas into the processing solution, the method and apparatus are cycled **313**, so dilution and auto-collection **314** are executed and when complete, islet processing ends **315**.

[0046] Figure 4 illustrates the automated islet separation apparatus process control valve logic **401** for the automated islet separation method and automated islet separation apparatus according to one preferred embodiment of the invention. After the automated method is started **301** (previously described in Fig. 3), the islet isolation apparatus initially fills **302** with physiologic process solution while process valves **104, 116, 126, 132, 154,** and **180** are open and process valves **115, 117, 125, 127, 131, 135, 137** and **153** are closed. During the circulation and rinse phase **303** of islet processing, process valves **116, 180, 126, 132, 139,** and **153** are open and process valves **104, 115, 117, 125, 127, 131, 135, 137,** and **154** are closed. When the process solution is drained **304** from the apparatus process valves **104, 116, 180, 126, 132,** and **137** are open and process valves **115, 117, 125, 127, 131, 135, 139, 153** and **154** are closed. The apparatus is refilled **305** with physiologic process solution with process valves **104, 116, 180, 126, 132,** and **154** open and process valves **115, 117, 125, 127, 131, 135, 137** and **153** closed. Pancreas addition **307** to the dynamic flow digestion chamber **120** is accomplished while process valves **115, 125, 126, 132, 139** and **153** are open and process valves **104, 116, 117, 180, 127, 131, 135, 137,** and **154** are closed. During pancreatic digestion, islet separation, and circulation **308** of the process fluid in the forward direction through the digestion chamber, process valves **116, 180, 126, 132, 139,** and **153** are open and process valves **104, 115, 117, 125, 127, 131, 135, 137** and **154** are closed. Throughout pancreatic digestion, islet separation, and circulation **308** of the process fluid in the reverse direction through the digestion chamber, process valves **115, 117, 125, 180, 127, 132, 139,** and **153** are open and process valves **104, 116, 126, 131, 135, 137** and **154** are closed. Auto-sampling and circulation **312** of the islet containing process fluid takes place while process valves **116, 180, 126, 132, 135, 139,** and **153** are open and process valves **104, 116, 118, 126, 128, 132, 137,** and **154** are closed. During dilution and auto-collection process valves **104, 116, 180, 126,** and **131,** are open and process valves **115, 117, 125, 127, 132, 135, 137, 139, 153,** and **154** are closed. Dissolved oxygen is displaced and removed from the process fluid by opening the helium control valve **149** and sparging the process fluid with helium from the helium tank **148**. Dissolved oxygen may be added to the process solution by opening the oxygen control valve **146** and sparging the process fluid with oxygen from the oxygen tank **147**.

[0047] Figure **5** illustrates the islet containing process solution flow in forward direction through the dynamic flow digestion chamber during pancreatic digestion, islet separation, and process solution circulation **309**, (previously described in Fig. **1,** Fig. **3,** and Fig. **4**) according to

one preferred embodiment of the invention. The process solution flows through the dynamic flow digestion chamber **120** in the forward direction while process valves **116**, **180**, and **126** are open and process valves **115**, **117**, **125**, and **127** are closed.

[0048] Figure **6** illustrates the islet containing process solution flow in reverse direction through the dynamic flow digestion chamber during pancreatic digestion, islet separation, and process solution circulation **309**, (previously described in Fig. **1**, Fig. **3**, and Fig. **4**) according to one preferred embodiment of the invention. The process solution flow through the dynamic flow digestion chamber **120** in the reverse direction is achieved while process valves **115**, **125**, **180**, **117**, and **127** are open and process valves **116** and **126** are closed.

[0049] All publications, patents, and patent documents are incorporated herein by reference, as though individually incorporated by reference. The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications might be made while remaining within the spirit and scope of the invention. The above descriptions of exemplary embodiments are for illustrative purposes. Because of variations that will be apparent to those skilled in the science, the present invention is not intended to be limited to the particular embodiments described above. Thus, various modifications of the above-described embodiments will be apparent to those skilled in the art or science. The present invention may also be practiced in the absence of any element not specifically disclosed. The invention may be uniformly applied pancreatic tissue containing islets of Langerhans with insulin producing beta cells harvested from animals and mammals either transgenic or non-transgenic. The scope of the invention is defined by the following claims.

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